

CLAIMS

- 00821453-051204
1. A method for obtaining transgenic plants expressing a protein with H₂O₂ producing activity, characterized in that it comprises the following steps of:
 - 5 (a) transforming plant cells with *Agrobacterium rhizogenes* containing a vector carrying a gene encoding a protein producing H₂O₂ in a context which allows its expression in the plant;
 - (b) selecting the transformants which contain and express this gene, by a peroxidase-based colorimetric test;
 - 10 (c) regenerating the plants from the roots selected and monitoring the expression of the plantlets obtained by a peroxidase-based colorimetric test;
 - (d) sorting according to phenotype and optionally carrying out a molecular analysis of the progeny of the transgenic plants, allowing the selection or the confirmation of transgenic plants obtained containing only the transgene and
 - 15 not the T-DNA specific to *A. rhizogenes*.
 2. A method of obtaining transgenic plants expressing a gene of interest and a gene encoding a protein with H₂O₂ producing activity, characterized in that it comprises the following steps of:
 - 20 (a) transforming plant cells with *Agrobacterium rhizogenes* containing a recombinant DNA comprising both a gene encoding the H₂O₂ producing protein and a gene encoding a protein of interest in a context allowing their expression in the plant;
 - (b) selecting the transformants which contain the gene encoding the protein
 - 25 of interest by a peroxidase-based colorimetric test;
 - (c) regenerating plants from selected transformants and monitoring the expression of the plantlets obtained by a peroxidase-based colorimetric test;
 - (d) sorting according to the phenotype and optionally carrying out a molecular analysis of the progeny of the transgenic plants in order to select or
 - 30 confirm the plants containing only the transgene and not the T-DNA specific to *A. rhizogenes*;
 - (e) purifying, where appropriate, the protein of interest produced.

3. The method according to one of claims 1 and 2, characterized in that the colorimetric test in step (b) is carried out on a sample of the root.
- 5 4. The method according to one of claims 1 and 2, characterized in that the colorimetric test in step (b) is carried out on a liquid medium for incubation after removing the agrobacteria.
- 10 5. The method according to any one of claims 1 and 2, characterized in that the colorimetric test in step (b) is carried out on the blot left by the transformed explants on an agar medium or on a membrane.
- 15 6. The method according to one of claims 1 and 2, characterized in that the selection in step (b) is carried out in the presence of a saturating concentration of substrate.
7. The method according to claim 6, characterized in that the saturating concentration of substrate is between 5 and 50 mM.
- 20 8. The method according to one of claims 1 and 2, characterized in that the colorimetric test in step (c) is carried out on a sample of plant tissue from the plantlets obtained.
9. The method according to claim 2, characterized in that the gene of interest is a gene of interest which is expressed at a late stage of development.
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10. The method according to any one of claims 1 to 9, characterized in that the plant cells are plant cells obtained from a major crop species chosen from rape, cauliflower, sunflower, wheat, corn, barley, tobacco, or from a culinary species such as tomato.
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11. The method according to any one of claims 1 to 10, characterized in that the plant cells are cells of cotyledons, hypocotyls or floral scapes.
12. The method according to any one of claims 1 to 11, characterized in that the plant cells do not endogenously produce oxalate oxidase.
13. The method according to any one of claims 1 to 12, characterized in that the protein of interest is an endochitinase.
14. Plant parts and/or plants which can be obtained by the method according to one of claims 1 to 13.
15. Plant parts and/or plants according to claim 14, characterized in that they are chosen from rape, cauliflower, sunflower, wheat, corn, barley, tobacco and tomato.

ADD A2 / add C1

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